A STUDY OF LIPIDS OF CHICKPEA

by

LEH-YEU LING

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Approved by:

Robert J. Robinson
Major Professor

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INTRODUCTION

Chickpea (Cicer arietinum, L.), a major food legume, is also known as "Bengal gram" and "chana" in India, "komos" in the Near East, and "garbanzo" in Latin American countries. Chickpea protein is known to contain more lysine than most of the other proteins of beans and pulses used in these areas. When added to chicken rations, the lysine of chickpea is superior to many other protein supplements. Its light tan color and bland odor and taste have very little detrimental effect on the physical characteristics or acceptibility of a blended food. Many studies using chickpea flour as a nutritional supplement have been reported. Lipids, in addition to protein, are also important dietary constituents, not only because of their high energy value but also because of the presences of fat-soluble vitamins and essential fatty acids. However, there is a dearth of published data on chickpea lipids. The purpose of this study was to tentatively fractionate and identify the components of chickpea flour lipids by silicic acid column, thin-layer chromatography (TLC) and gas-liquid chromatography (GLC).

REVIEW OF LITERATURE

Botanical Classification of Chickpea

The chickpea plant was classified by Altschul (1958) as follows:

Family: Leguminosae

Subfamily: Papilionatae

Tribe: Vicieae

Genus: Cicer

Species: Arietinum

The most common varieties of Cicer arietinum yielded seeds which were usually whitish or slightly brown. Others produced reddish or dark seeds. The chickpea might vary considerably in size and weight. The plant was commonly grown for seed.

Winton and Winton (1935) also studied the morphology of chickpea.

Distribution and Production of Chickpea

Chickpea was probably a native of regions south of the Caucasus and Caspian Sea, where it has been cultivated since prehistoric times. It was introduced early into India, and somewhat later into all the Mediterranean countries. From Spain it was introduced into Spanish colonies (Winton and Winton, 1935).

The production of chickpea in 1955 was 6.7 million metric tons, or around 28% of the total legume seed produced (Altschul, 1958). Whereas the production of chickpea was large, its world distribution was restricted. India and Pakistan produced and consumed around 90% of the world production. The rest of the seed produced was used by several Mediterranean countries and to some extent, by some of the Latin American republics. In the American continents, Mexico was the leading producer. It produces two types of chickpeas. The large garbanzos from the north constituted the export crop; the small chickpea grown in central Mexico accounted for about 60% of the national production. In Egypt both small and large chickpea were produced.

Gross Composition of Chickpea

Cozzi (1939) studied chemical and physical characters of the chickpeas of Italian East Africa. He reported that ripe fruits contained water 10.80-12.57, ash 3.11-3.82, protein (N x 6.25) 15.66-18.15, non-protein N-containing matter 0.95-2.14, fat 3.22-5.20, cellulose 4.26-7.13, soluble sugars 2.82-6.72, starch 36.22-38.62%.

Intengan et al. (1954) made analyses of 167 samples of indigenous food plants and food products in the Philippines for moisture, N, crude fiber, fat ash, Ca, P, Fe, carotene, thiamine, riboflavin, ascorbic acid, and niacin. They found chickpea might be considered one of excellent or good source of two or more nutrients.

Diaz and White (1953) gave the content of protein, fat, carbohydrate, fiber, ash, Ca, Fe, thiamine, riboflavin, and nicotinic acid in beans, lentils, garbanzos, and other legumes consumed in Peru.

The gross chemical composition of chickpea, according to the data summarized by Aykroyd and Doughty (1964) was:

Water	(g)	11.0
Protein	(g)	20.1
Fat	(g)	4.5
Carbohydrate		
total by difference	(g)	61.5
fiber	(g)	4.9
Ash	(g)	2.9
Calcium	(mg)	149.0
Iron	(mg)	7.2
Vitamin A value	(I. U.)	300.0
Thiamine	(mg)	0.40
Riboflavin	(mg)	0.18
Niacin	(mg)	1.6
Ascorbic Acid	(mg)	5.0

(per 100 g. edible portion)

Lipids in Chickpea

The physical and chemical properties of the extracted lipid by ether of chickpea are as follows (Winton and Winton, 1935):

Specific gravity (at 15°C)	0.9369-0.9376
Refractive index (at 25°C)	1.4744-1.4747
Solidification point	-19.5C
Saponification number	240
Iodine number	110-119
Reichert-Meissl number	4.51
Polenske number	1.1
Hehner number	91.6
Ester number	239.5
Melting point of fatty acids	25°C
Iodine number of fatty acid	129
Acid number	0.3 to 0.5
Unsaponifiable matter	0.48%

Bhandari et al. (1950) reported the physical and chemical constants of the chana and Kabuli chana oils as follows:

	Chana oil	Kabuli chana oil
Specific gravity (at 40°C)	0.9356	0.9301
Refractive index (at 38°C)	1.4845	1.4825
Saponification value	184.6	185.4
Acid value	2.4	2.6
Iodine value (Wijs)	111.7	113.2
Reichert-Meissl value	0.61	0.60
Polenske value	0.38	0.40
Unsaponifiable matter, %	3.4	4.0
Sterol content, % (on the weight of the oil)	2.9	3.2

They concluded that the physical properties and chemical composition of the fixed oils extracted from the two varieties of Cicer arietinum, L., viz., chana and Kabuli chana, were nearly identical.

Date (1955) studied the antioxidant property of several legume flours. He found chickpea flour had the lowest phosphatide content, 0.289%.

Talwalkar et al. (1966) found that phosphatide prepared from Cicer arietinum consisted predominately of choline-containing lecithins. They also found that the stabilizing property of plant lecithins on water-oil emulsions was comparable to that of egg yolk lecithin.

Saponins, carbohydrate-containing triterpenoids or steroids, were found in chickpea seed by Applebaum et al. (1969). They also suggested saponins as possible factors of resistance of legume seeds to the attack of insects.

Carbohydrates

a. Varieties

Parihar (1954) studied the changes in saccharides of stored pulses, Cicer arietinum, Lens esculenta, Cajanus indicus, Phaseolus aureus. He found fructose, galactose, and glucose were more abundant in the stored pulses than in fresh material. Nigam and Giri (1961) reported that sugar composition of chickpea, in grams per 100 grams seed, were: sucrose 2.4, raffinose 1.0, stachyose 2.5, verbascose 4.2.

El-Hanafy and Taha (1963) isolated an arabinoglucoglycan, composed of L-arabinose and D-glucose in a ratio of about 3:7 in 0.8-1.2% yield from the seed of Cicer arietinum.

Pant and Kapur (1963) reported the total soluble carbohydrate of Cicer arietinum was 6.7%; glucose and sucrose were also present in Cicer arietinum.

b. Starch

Sarin and Qureshi (1938) found the creamy white variety of gram (Cicer arietinum) yielded 21% of pure white starch. They also found the gram starch granules were of medium size, resembled maize starch in general appearance and viscosity, were suitable for sizing cotton, wool, and silk cloth.

Patel et al. (1960) undertook an investigation on the specific rotation of 16 cereal and legume starches. They found the specific rotation for chickpea was 201. They also reported genetic factors and growth conditions had little influence on the specific rotation of starch.

Tolmasquim et al. (1965) studied the Brabender viscosity of chickpea starch. They found variation of the pH of a 5 g/100 ml aqueous suspension showed maximum viscosity at pH 3.2-4.0. They also suggested that chickpea starch was cross-bonded type.

Correa et al. (1965) studied the swelling power and the percentage of solubles of chickpea starch at pasting temperatures between 60^{o} and 90^{o} at 5^{o} intervals. They got a curve similar to that of sorghum and natural maize starches. They also found defatting caused an increase in these values, suggesting that the presence of fat is one of the factors responsible for the restricted swelling of chickpea starch.

Srivastava et al. (1970) undertook physicochemical studies on some starches. They tabulated the results of granule characteristics and analysis of chickpea starch as follows:

Granule Characteristics of Chickpea Starch

Source	Chenna (Cicer arietinum)
Shape	Round
Size (micron)	3-4
Hilum	Centric, sometimes fissured
Striae	None
Intensity of polarization	High
Gelatinization temp. range (^{o}C)	71-74

Analysis of Chickpea Starch

Yield (%) on dry weight of seed kernels	62
Ash (%)	0.193
Protein (%) (N_2 x 6.25)	6.89
Fat (%)	0.74
Phosphorus (%)	0.057
Iodine Affinity (%)	5.65

Proteins

Massieu et al. (1950) studied essential amino acid content in several Mexican seeds. They found that nitrogen content of the defatted, dried material was between 3.73-4.37 percent. The lysine content of all seeds was lower than that of whole-egg protein (WEP), that of chickpea, pea and broad bean was greater than that of soybean. Leucine content was higher than in WEP in chickpea (14.8%). The phenylalanine value for chickpea (5.0%).

Lal (1950) made a microbiological assay of amino acids in major articles of food in India, gram (Cicer arietinum) and ragi (Eleusine coracana). He

showed the results of analysis of whole-grain samples from Bihar as follows: 0.21 and 0.08 percent tryptophan, 2.4 and 0.14 percent lysine, 1.2 and 0.13 percent arginine, 0.00 and 0.00 percent threonine, 4.19 and 1.28 percent total N and 2.5 and 2.8 percent ash (all on dry-weight basis) and 12.1 and 14.6 percent moisture in gram and in ragi, respectively. He also concluded that the amino acids estimated were present in quantities which made gram and ragi useful foods for maintenance of health and that gram was also useful for growth.

Altschul (1958) summarized the essential amino acid content of protein of chickpea as follows:

Protein content % (dry basis)	23.5
Amino acids (g. per 16 g. of nitrogen)	
Isoleucine	5.9
Leucine	8.5
Lysine	6.5
Methionine	1.5
Phenylalanine	6.5
Threonine	4.9
Tryptophan	1.0
Valine	6.2
Arginine	6.9
Histidine	3.0

Colobrado *et al*. (1959) studied the amino acids in the proteins of various edible legumes in Argentina. He obtained the following results: The phenylalanine content in products studied varied from 4.82 in lentils to 6.24% in chickpea.

Lain et al. (1963) reported the crude protein content of the seed (N x 6.25) of black chickpea was 18.28%.

Alamo (1963) studied the content of proteins, and lysine in these vegetables, broad beans and chickpeas from various parts of Chile. He found the chemical method gave lower results of lysine content than the microbiological method.

In Chile, Neisser (1964) reported the chickpeas averaged 0.132% tryptophan (by weight) and the broad beans 0.12%.

Torres and Godoy (1967) investigated the isoleucine, leucine, and phenylalanine content in Chilean legumes. They found the amounts of leucine and phenylalanine were higher in chickpeas than in lentils, and of isoleucine nearly in the same amounts in both. They also found that if compared with the Food and Agriculture Organization of the United Nations (F.A.O.) provisional amino acids pattern, the amount of isoleucine was 87% of the standard. Leucine was 1.1- and 2.2- and phenylalanine 2.1- and 2.5-fold greater than the standard, in lentils and chickpeas, respectively.

Schlack (1966) evaluated microbiologically the content of methionine, threonine, leucine, and isoleucine in Chilean chickpeas. He found in g. % and in mg/g. N, the amino acid content found was comparable with that of the Reference Standard of the Food and Agriculture Organization, and in fact, quantitatively, very superior in leucine and isoleucine.

Sanzana and Godoy (1967) found the amounts of threonine, valine, and methionine were higher in chickpeas than in lentils. Comparison with the Food and Agriculture Organization and the United Nations (F.A.O.) standard protein showed that in chickpeas, the amounts of threonine were 2.7-fold higher, while valine and methionine were 78% and 39% of standard levels.

Leonov (1966) investigated the variations in protein contents in seeds of some Cicer arietinum varieties. He found the protein N, the main fraction of which was globulin fraction, was 75-85% of the total N; globulin fraction was separated electrophoretically into 5 components, the components of which depended on the variety and growing conditions.

Leonov (1968) found the globulin fraction constituting the main part of the proteins in chickpea cotyledon was a complicated system consisting of 4 or 5 components. In order to obtain a more perfect separation, gradient-elution column chromatography on DEAE cellulose was used, and up to 11 peaks, could be identified.

Vitamins

Rudra (1938) studied the vitamin C content of germinated cereals and pulses. He found the vitamin C content of germinated chickpea was 6.85 p.p.m. Giral and Alvarez (1943) presented vitamin C content of Mexican legumes and other vegetables. The value for vitamin C of chickpea was 1.59 mg. %; 0.12 mg. % for dehydro-ascorbic acid. Rudra (1943) estimated the ascorbic acid contents of the cereals and pulses. The value obtained for chickpea was 10.31 mg m/gm. Chandra and Arora (1968) made an estimation of ascorbic acid on 40 different indigenous and introduced chickpea varieties. They found vitamin C content varied from 2.5 mg and 6.0 mg per 100 g seed material, with the green-cotyledonous Green Gram Bijiapur and Green Gram Ferozepur having the maximum level.

Urrutia (1949) found the nicotinic acid values in mg. % were: chickpea 1.5, white bread 4.5, whole-wheat bread 6.0, chochoca 0.4, camote (sweet potato) 0.1, carob-tree flour 0.3, Vicia faba (field bean) flour 1.5.

Asenjo (1954) reported the chickpea contained 1251 µg pantothenic acid per 100 g edible portion.

Goldberg et al. (1945) made a survey of thiamine content of beans and other legumes in Africa. They found that most legumes were far superior to cereals as dietary sources of thiamine. They also found the mean thiamine values of chickpea were 3.50 µg/g. Fabriani and Spadoni (1947) studied thiamine content of Italian vegetables and fruits. They found dry chickpea contained 400 µg per 100 g of the eatable part. They also found cooking of vegetables caused loss of thiamine content. Iengar et al. (1955) investigated the thiamine contents of a few Indian leguminous seeds during germination. They reported the thiamine content of Bengal gram, pea, and horse gram dropped when germination started; but when the seed began to grow, the thiamine content increased. They also reported seeds germinated and growing in light had a higher thiamine content than those grown in darkness.

Carasco (1954) evaluated riboflavin in seeds of germinated legumes. They found chickpea contained 170 µg per g. dry weight.

Banerjee and Chatterjea (1964) studied folic acid of Indian dietary articles. They found ungerminated Bengal gram contained 12.4 µg folic acid activity per 100 g, 18.4 for germinated Bengal gram.

Nazir and Magar (1963) studied tocopherol content of pulses. They found chickpea contained 1.51-1.58 mg vitamin E per 100 g chickpea; 75.7-79.1 for chickpea fat.

Altschul (1958) summarized the vitamin contents in chickpea as follows:

	mg/100 g whole seed (dry basis)
Thiamine	0.81
Riboflavin	0.19
Niacin	1.68
Ascorbic acid	0.72

extracts could be completely destroyed at 100°C. He also found cyanase activity following germination increased daily to a maximum of 6471 x 10^{-4} in 12-day-old seedlings and decreased to 4507 x 10^{-4} in 15-day-old seedlings.

Hadi (1966) studied the changes in nucleic acids and nucleases over a 6-day germination period of Cicer arietinum. He found that during the first 3 days of germination a 25% decline in both DNA and RNA was noted which was associated with an increase of DNase and RNase along with a steady rise in the levels of free ribonucleotides.

RNase, DNase, phosphomonoesterase, and 3-nucleotidase were separated and purified 210, 350, 10, 9-fold, respectively, from germinating Cicer arietinum seedlings by $(NH_4)_2SO_4$ precipitation and column chromatography. (Hadi et al., 1968). The presence of a RNase inhibitor in seedling homogenates was also indicated. Phosphomonoesterase was characterized as an acid phosphatase. In its pH optimum, specificity to substrates and sensitivity to inhibitors, the 3-nucleotidase resembled 3-nucleotidase from other plant sources, whereas the RNase was dissimilar.

Nair and Vaidyanthan (1961) demonstrated tryptophan synthetase activity in the cell-free extracts of the chickpea resting seeds. They also found the optimum pH of the reaction was 5.5, and the Km value for indole at a constant serine concentration of 10^{-4}M was 0.57 x 10^{-4} M.

In 1964 Nair and Vaidyanthan carried out the study of the purification and properties of tryptophan synthetase of chickpeas. They found that a 220-fold purification of the enzyme with 44% recovery of the activity was achieved They also found this enzyme was of a competitive type.

Minerals

Winton and Winton (1935) summarized the data on mineral constituents of chickpea as follows:

K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	SO_3	SiO_2	Cl	Mn	Cu
%	%	%	%	%	%	%	%	%	%	%
24.60	1.29	4.45	19.98	2.42	39.56	3.38	2.85	0.71	0.00166	0.0010

Giral and Castillo (1953) made the determination of copper in garanzos. The value obtained was 1.898 p.p.m.

The data quoted by Altschul (1958) on mineral content in chickpea follow: calcium 115, phosphorus 438, iron 9.67 mg per 100 g whole seed (dry basis).

Roychowdhury et al. (1962) studied minor mineral contents on 62 foods commonly in Bihar by a wet-oxidation process. They found Cu was 49.1 mg/kg (dry basis) in Bengal gram.

Guttikar et al. (1966) had analyzed a total of 27 Indian foodstuffs consisting of 3 pulses, 3 leafy vegetables, 18 other vegetables, and 3 fruits for their copper and magnesium contents. They found legume Bengal gram was one of the richest of sources of both elements (copper: 990 µg/100 g; Mg: 108 mg/100 g).

Pigments

Wong and Mortimer (1965) made an investigation of flavonoid constituents of Cicer arietinum. They found isoliquiritigenin, isoliquiritigenin 4'-glucoside 3,4',7-trihydroxylflavone, daidzein, pratensein, Pcoumaric acid, 3,4',7-trihydroxylfavanone (garbanzol) and biochanin 7-glucoside in chana seedlings.

Hoesel and Barz (1970) studied the flavonoids of Cicer arietinum. They isolated the flavonols kaempferol, quercetin, and isorhamnetin, together with the isoflavone pratensein from above ground parts of Cicer arietinum. They also found the main kaempferol glucosides present were kaempferol of 3-β-glucoapioside.

Proteinase Inhibitor in Chickpea

Borchers and Ackerson (1947) made an investigation of some legume seeds other than soybean and other seeds for the possible presence of a trypsin inhibitor. The trypsin inhibitor was found in chickpea.

Trypsin inhibitor activity was also found in Bengal gram by Sohonie and Bhandarkar (1954). After extraction from chickpea, this inhibitor was 85.7% destroyed on heating in a boiling water bath for 60 minutes. The percent destroyed at 15, 30, and 45 minutes was 21.4, 50.0, and 64.3. respectively. Autoclaving at 15 lb. pressure for 30 minutes destroyed 90.4% of the inhibitor.

Proteinase inhibitor in the seeds of Cicer arietinum was investigated by Abramova and Chernikov (1964). The antitryptic and antichymotryptic activity was found in Cicer arietinum. However, no antipeptic activity was found.

Chernikov et al. (1966) reported moderate trypsin and chymotrypsin inhibitors in chickpea. The inhibitors were completely inactivated by heating for 15 min. at 100°C in 0.1 N Na_2CO_3; however, in 0.1 N HCl under the same conditions their activity was preserved.

Nutritive Value of Chickpea

Nutritive value of Cicer arietinum and six other pulses was studied by Esh and Som (1952). Among them, total protein content varied from 21.7 to 31.7%, total lipid from 1.0 to 5.2%, and ash from 2.3 to 4.3%. Digestibility figures were fairly uniform for the samples, and biological value was 64.6% for Cicer arietinum. Heating for 30 min. at 15 lb. pressure did not improve the nutritive value of Cicer arietinum. Supplementation with methionine led to improved growth with all the material.

Adolph et al. (1955) found that protein efficiency ratio (PER) of raw chickpeas and average weight gain of rats were 1.4 $\pm$ 0.24, and 30.4 g,

respectively, when the protein intake was 20.6% of diet. On the other hand, the PER of cooked chickpeas and average gain in weight were 2.05 ± 0.10, and 49.1 g., respectively, at approximately the same protein level.

Srikantia and Gopolan (1960) found that the incorporation of chickpeas in a vegetable protein diet was effective in controlling the clinical manifestation of protein malnutrition in children.

Ganapati et al. (1961) showed that two foods were acceptable, tolerated, improved the health, and augmented the growth of children. One of these foods consisted of: (in parts) roasted chickpea flour, 80; and skim milk powder, 20; the second one consisted of defatted peanut flour, 75; roasted chickpea flour, 25; lucerne powder, 1; and $Ca_3(PO_4)_2$, 1.

Three common Indian pulses, Cicer arietinum (chickpea), Cajanus cajan (pigeon-beans), and Phaseolus radiatus (adjuki beans) were studied with respect to composition, digestibility, protein value and nutritional and biological value. Chickpea had the highest biological value (1.3) and highest protein value (61.10) among the 3 pulses (Pant and Kapur, 1963).

In the Middle Eastern countries, a protein-rich mixture of vegetable foods consisting of 47% autoclaved chickpeas, 35% defatted sesame flour, and 18% heat-processed low-fat soybean flour was available (Guggenheim and Szmelcman, 1965). Its biological value was 74 and its protein efficient ratio (assessed on young rats in 28 days' assays) was 2.90.

Male Wistar rats (60 g.) were fed for 25 days with 1 of 5 diets: (1) 84.5% ground wheat plus 15% protein, 3% of which was from wheat gluten (basal diet), (2) cooked chickpeas substituted for wheat gluten in the basal diet, (3) cooked lentils substituted for wheat gluten in the basal diet, (4) a 50% mixture of lentils and chickpeas substituted for wheat gluten in the basal diet, and (5) powdered milk substituted for wheat gluten in the basal diet.

The weight gain per gram ingested protein with diets 2, 3, and 4 was 2-fold greater than that with diet 1 which was 0.7. It appeared that the seed proteins of chickpeas were useful dietary complements of cereals (Kande, 1967).

Shehata and Fryer (1970) investigated the protein quality of Egyptian bread made from wheat flour supplemented with chickpea flour. In their investigation, dough was made from combinations of 80% extraction hard red winter wheat flour and 0, 5, 10, 15, and 20% chickpea flour. Protein quality of the bread was determined by studying weight gain, feed consumption and protein efficiency ratio (PER) of rats. Percent weight gain of rats fed 20% chickpea flour was significantly higher than that of rats fed diets with 0, 10, and 15% chickpea flour. There were significant increases in feed consumption with the 15% and 20% chickpea flour which had measured PER's of 1.46 and 1.56, significantly better than that of rats fed diets with 0% chickpea flour, which was 1.18.

MATERIALS AND METHODS

The chickpea seed imported from India was decorticated and milled to pass 10 XX sieves (opening size range: 130-135 microns) in the experimental facilities of the Department of Grain Science and Industry at Kansas State University.

The chickpea flour was stored in a cold room to prevent enzymatic lipolysis and other deleterious effects which may occur at room temperature.

Extraction of Lipids

Lipids of chickpea flour were extracted by the methods as follows:

a. Acid hydrolysis method. The procedure was described in Cereal Laboratory Methods (1957), and further modified by Tsen et al. (1962). The results obtained were expressed as percentage of lipids.

b. Petroleum ether and ethyl-alcohol extraction. Samples of 5 grams were extracted overnight (14-16 hrs) with petroleum ether (Ligrosine, Mallinckrodt Chemical Works, St. Louis, Mo.) or ethyl alcohol in a Goldfish extraction apparatus by the direct method for grain and starch feed (AOAC methods, 1965).

c. Water-saturated n-butanol extraction. Samples of 15 g were extracted with water-saturated n-butanol by the following procedure (Pomeranz et al., 1966) with some modification. The lipids were subsequently extracted in a Laboratory Stein Mill (The Fred Stein Laboratories, Atchison, Kansas) for 2 min. with 1 min. intervals with 100, 50, 50, 50, and 50 ml instead of 100, 50 and 50 ml water-saturated n-butanol. The combined extract was decanted, filtered and evaporated almost to dryness under reduced pressure in a rotary flask evaporator at 40-45°C. The extract was kept under vacuum in

a desiccator for about two days over P_2O_5 at 4°C. It was further extracted three times with petroleum ether. The combined extract was evaporated under reduced pressure at about 40°C. The crude lipids were dissolved in 80 ml of chloroform-methanol mixture (2:1 v/v). 17.5 ml of 0.04 percent calcium chloride was used to extract some impurities followed by two extractions with 10 ml of each of aqueous 0.02 percent calcium chloride solution. The volume of the lipid was made up to 50 ml with chloroform. 5 ml portions of lipid solution were dried in an air oven at 85-95°C to constant weight. The total lipids were determined by residue weight. The remaining lipid solution was concentrated under vacuum.

Silicic Acid Column Chromatography

Silicic acid column (2 x 15 cm) was used. Lots of 30 g of silicic acid (Sigma Chemical Company, St. Louis, Mo.) were washed with distilled water and dried at 120°C for 4 hr. The silicic acid was then washed twice with 60 ml of chloroform-methanol mixture (15:1), and once with 60 ml of chloroform-methanol mixture (7:1), and finally with 80 ml chloroform. The silicic acid slurry was transferred to column. The concentrated lipid solution was applied to the column. The neutral lipids were eluted with 140-150 ml chloroform and the polar-lipids with 150-170 ml methanol. Completion of elution was checked by thin-layer chromatography. Fractions were concentrated to 100 ml under vacuum. 10 ml of solution was withdrawn and dried to determine neutral and polar-lipid content. The remaining solution was evaporated under reduced pressure to remove the solvent (Pomeranz et al., 1966).

Thin-layer Chromatography

Glass plates (20 x 20 x 0.38 cm) were coated with a 250 mμ layer of silica gel G for TLC, according to Stahl (E. Merck, A. G. Darmstadt, Germany) with

a commercial spreader (C. A. Brinkman Co., Great Neck, N. Y.) (Stahl, 1969). The plates were activated for 3 hrs. at 130°C and allowed to cool in a desiccator (Daftary et al., 1965).

The silicic acid column chromatography fractionated lipids were applied at a level of 20-100 µg per spot. The chromatographic solvents used for one-dimensional ascending development were hexane-diethyl ether-acetic acid mixture (70:30:2) for neutral lipids (Mangold, 1961) and chloroform-methanol-water mixture (60:30:5) for polar-lipids (Wagner et al., 1961).

The chromatography chamber was lined with filter paper in order to insure saturation of the chamber space with solvent vapors. The solvent vapor in the chamber was saturated in 12 hours (Mangold, 1961). Length of run was 15 cm. Time of run for neutral and polar-lipids 34 min. and 70 min., respectively. The plates were air-dried for half an hour.

The plate with neutral lipid fractions was made visible by exposure to iodine vapor (Sim and Larose, 1962). Bright yellow to brown spots on a white background were given by unsaturated compounds. After evaporation of iodine, the plates were sprayed with chromic-sulfuric acid (Rouser et al., 1964), then charred by heating the plate in an oven at 180°C for 25 min. (Privett and Blank, 1963).

The polar-lipids were sprayed first with ninhydrin reagent (Lepage, 1964) for the detection of free amino groups. The sprayed plate was heated for a few min. at 100°C. Purplish pink spots were shown in the presence of free amino group containing lipids. The plate was again sprayed with molybdenum spray. Blue spots on white or light blue-gray background were shown immediately in the presence of any phosphorus-containing lipids (Diffmer and Lester, 1964). Orange spots on a white or light yellow background were shown in the presence of choline-containing phospholipids by the modified

Dragendorff spray (Bregoff et al., 1953). Glycolipids were detected as blue-gray spots on a white background by diphenylamine reagent followed by heating at 105°C for 30 min. (Randerath and Kurt, 1963).

Thin-layer chromatography fractionated lipids were tentatively identified by the use of specific sprays and by comparing R_f value with those of pure compounds.

Neutral lipids, 1,3-diolein, 1,2-diolein, trilinolein and cholesteryl oleate (Applied Science Laboratories, State College, Pennsylvania) and linoleic acid (K and K Laboratories, Plainview, N. Y., Hollywood, Calif.) were used as reference.

For polar-lipid tentative identification, DL-α lecithin (synthetic), phosphatidyl ethanolamine, phosphatidyl inositol (Applied Science Laboratories, State College, Pennsylvania), d (+) raffinose (Pfanstiehl Chemical Co., Waukegan, Illinois), and sucrose (J. T. Baker Chemical Co., Phillipsburg, N. J.) were used as reference.

The plates were photographed after charring with chromic-sulfuric acid.

Preparation of Fatty Acid Methyl Esters for Gas-Liquid Chromatography

The transesterification procedure for preparation of fatty acid methyl ester of Jamieson and Reid (1965) was used and modified as follows:

1. Put 2 to 3 drops of lipid solution into test tube and add approximately 10 ml of 1% KOH in absolute methanol and transesterify by heating at 70°C for 30 min.
2. Recover esters by acidification with glacial acetic acid, add a few ml of petroleum ether, then HOH. Recover petroleum ether solution. Repeat extraction with petroleum ether.
3. Combine petroleum ether solutions, wash with HOH, and dry with anhydrous sodium sulfate.

4. Recover petroleum ether solution and evaporate very nearly to dryness under a stream of nitrogen.
5. Determine fatty acid composition by gas-liquid chromatography.
6. In order to identify fatty acids from chickpea flour lipids, a standard mixture was used which was obtained from Dr. Klopfenstein, Department of Biochemistry, Kansas State University. This standard contained capric, lauric, myristic, palmitic, stearic and arachidic fatty acids.
7. The characterization of unsaturated fatty acid esters of chickpea flour lipids was made according to the method of Hofstetter et al. (1965).

Gas-Liquid Chromatography

Fatty acid methyl ester was analyzed by gas-liquid chromatography (GLC) with a Barber-Colman 5000 gas chromatograph equipped with flame ionization detector and 6 ft. x 1/8 in.o.d. glass U-tube packed with 7.5% diethylene glycol succinate (DEGS) on 60-80 mesh AW was 190°C and the carrier gas was high purity argon used at a flow rate of 40 ml/min. (Klopfenstein, 1971).

Determination of Nitrogen and Phosphorus in Extracted Lipids

Total nitrogen determination: Total nitrogen in extracted lipids was determined by micro-Kjeldahl procedure (AOAC method, 1965). 5 ml of lipid solution containing 51-83 mg. extracted lipid was transferred to 30 ml digestion flask. Added 1.9 g K_2SO_4, 40 mg HgO, and 3 ml H_2SO_4. Added boiling chips, digested 1 hr. after all H_2O was distilled and acid came to true boil. Cooled, added minimum quantity of H_2O to dissolve solids, cooled, and placed thin film of vaseline on rim of flask. Transferred digest to disstillation apparatus and rinsed flask 5 or 6 times with 1-2 ml portions H_2O. Placed

125 ml Erlenmeyer flask containing 5 ml standard H_3BO_3 solution. Added 10 ml NaOH-$Na_2S_2O_3$ solution to still, collected ca 15 ml distillate and diluted to 50 ml. Titrated to gray end point or first appearance of violet. Made blank determination and calculated % N = (ml HCl -ml blank) X normality X 14.007 X 100/mg sample.

Phosphorus content in the extracted lipids was determined by the method of Harries and Popat (1954). 0.0200-0.0335 gm of extracted lipid was put into 30 ml digestion flask. Added 1.0 ml of 70-72% $HClO_4$, one drop of concentrated HNO_3 and one or two glass beads and heated gently until the oxidation reaction subsided. Added 2 more drops of concentrated HNO_3 and heated until the digestion was complete and white fumes of $HClO_4$ appear. Cooled the digest and transferred it to a 25 ml volumetric flask. Diluted somewhat with water, added 1.0 ml of molybdate solution, and mixed. Then added, without delay, 2 ml of elon solution and diluted the mixture of 25 ml.

Prepared the blank by diluting a mixture of 1.0 ml of perchloric acid, 1.0 ml of molybdate solution, and 2.0 ml of elon solution to 25 ml.

Set the spectrophotometer at 820 mμ and adjusted the instrument to read zero with the blank as a reference. Read the optical density of the sample and compared with a reference curve prepared by use of the standard phosphorus solution. Reading was taken at the same interval (25 min.) after addition of the reducing agent.

Physical and Chemical Properties of Chickpea Oil

Chickpea oil was extracted from chickpea flour with Skelly F in a Soxhlet extraction apparatus, and used for the following physical and chemical properties determination. Saponification Value: Determination of saponification value of chickpea oil was made by colorimetric titration method.

(AOCS official and tentative methods, Method Cd 3-25, 1958) 5 grams of chickpea oil was put into the 250 ml round bottom flask. Added 50 ml of the alcoholic potassium hydroxide with a pipette and allowed the pipette to drain for a definite period of time. Prepared and conducted blank determinations simultaneously with the sample and similar in all respects. Connected an air condenser, at least 650 mm long, and boiled gently but steadily until the sample was completely saponified. After the flask and condenser have cooled somewhat, but not sufficiently for the contents to jell, washed down the inside of the condenser with a little distilled water. Disconnected the condenser, added about 1 ml of indicator (1% phenolphthalein in ethyl alcohol) and titrated with 0.5 N hydrochloric acid until the pink color has just disappeared.

Saponification Value = 28.05 (titration of blank-titration of sample)/ weight of sample

Iodine Value: The iodine value was determined by the Hubl method (Mehlenbacher, 1960) placed 0.250 gm of the chickpea oil in a dry 500 ml glass-stoppered flask. Added 20 ml of the Hubl reagent, stoppered the flask, mixed the contents, and allowed to stand in the dark for 12 hrs. Added 20 ml of 30% potassium iodine solution, and added about 300 ml of distilled water and titrated with 0.1 N sodium thiosulfate solution using starch solution as the indicator. Prepared and conducted blank determinations simultaneously with the sample. Calculated the iodine value using the following equation:

Iodine Value = (B-S) X N X 12.69/weight of sample

B = Titration of blank

S = Titration of sample

N = Normality of sodium thiosulfate solution

Unsaponifiable Matter: The unsaponifiable matter was determined by petroleum ether extraction method. (AOCS official and tentative methods, Method Ca 6a - 40, 1958) 5 grams of chickpea oil was put into a 300 ml round bottom flask. Added 30 ml of alcohol and 5 ml of aqueous potassium hydroxide (50% by weight). Boiled gently but steadily under a reflux condenser for one hour or until completely saponified. Transferred the saponified mixture to a 500 graduated cylinder and washed the saponification flask with 95% alcohol until the total contents of the cylinder reached the 40 ml mark. Completed the washing, using warm and then cold distilled water until the total volume in the cylinder was 80 ml. Then washed out the flask with a little petroleum ether and added it to the cylinder. Cooled the cylinder and contents to room temperature (20-25^{o}C) and then added 50 ml of petroleum ether.

Inserted the stopper and shaked the cylinder vigorously for at least one minute, then allowed it to stand until both layers were clear. Drained off the lower aqueous phase and the petroleum ether fractions were drawn into and accumulated in a 500 ml separatory funnel. Repeated the extraction using 50 ml portion petroleum ether each time, at least 6 more times, shaking vigorously with each extraction.

Washed the combined extracts in the separatory funnel three times with 25 ml portions of 10% alcohol in distilled water, shaking vigorously and drawing off the alcohol layer after each washing. Transferred the petroleum ether extract to a tared beaker and evaporated it ot dryness on a water bath under a gentle stream of clean, dry air. Completed the drying to constant weight, preferably in a vacuum oven at 75-80^{o}C. Cooled in a desiccator and weighed. After weighing, took up the residue in 50 ml of warm (about 50^{o}C) 95% alcohol containing phenolphthalein (1% in 95% alcohol) indicator and previous neutralized to a faint pink color. Titrated with 0.02 N NaOH to the same color.

Grams of fatty acids in the extract = ml of 0.02 N NaOH X 0.0056

% unsaponifiable matter = (wt of residue-wt of fatty acid) X 100/wt of sample

Refractive Index: The refractive index was determined by means of refractometry as described on AOCS official and tentative methods (method Cc 7-25, 1958). Chickpea oil was filtered through filter paper to remove any solid impurity and the last traces of moisture. Adjusted the temperature of the refractometer to the desired temperature (25°C); then placed several drops of the sample on the lower prism. Closed the prisms and tightened them firmly with the screw-head. Allowed to stand for one to two minutes or until the sample came to the temperature of the instrument. Adjusted the instrument and light to obtain the most distinct reading possible and then determined the refractive index.

RESULTS AND DISCUSSION

Extraction of Lipids

The results of lipid extraction by four different methods were shown in Table I. It was found that water-saturated n-butanol was the most effective solvent system for the extraction of lipids from chickpea flour. It was also reported by Mecham and Mohammad (1955) and by Morton (1950) that this solvent system was outstandingly efficient in the removal of lipids from biological materials. Acid hydrolysis method was also effective, but it might cause the hydrolysis of some compounds other than lipids and result in unpredictable results. Petroleum ether and ethyl alcohol method was less effective. However, the difference in extractibility between these two methods was not significant.

Silicic Acid Column Chromatography of Water-saturated n-Butanol Extractable Lipids

Lipids extracted by water-saturated n-butanol were fractionated by silicic acid chromatography. The result was shown in Table II. The level of neutral lipids was ninety-one percent of the total lipid. If the lipid was not washed by a dilute calcium chloride solution before silicic acid column chromatography, the neutral lipid was decreased to seventy-six percent. For the polar-lipids, it was six percent for the former, and nineteen percent for the latter. The less polar-lipid content in the former case was considered due to the removing of non-lipid polar impurities during calcium chloride washing. This result was in good agreement with the finding of Daftary et al. (1966). Thin-layer chromatography chromatograms of both washed and unwashed polar-lipid were shown in Fig. III, IV. It was apparent that sucrose and raffinose were eliminated by calcium chloride washing. However, there was no difference in the TLC chromatograms of silicic acid column fractionated

neutral lipids with or without calcium chloride washing before column chromatography (Fig. I, II).

Thin-Layer Chromatography

The thin-layer chromatography chromatograms of the lipids obtained by different treatment were shown in Fig. I - IV. The fractionation of neutral lipids revealed that triglyceride was the major component with small amount of 1,3-diglyceride and 1,2-diglyceride. Traces of free fatty acid and sterol ester were also found. For the polar-lipid fractions, components were tentatively identified and listed in the order of decreasing R_f value.

A) Possible phospholipids according to the specific reaction (molybdenum reagent +)

B) Possible glycolipids according to the specific reaction (diphenylamine reagent +)

C) Phosphatidyl ethanolamine

D) Lecithin

E) Phosphatidyl inositol

F) Sucrose

G) Raffinose

Fatty Acid Composition of Chickpea Flour Lipid as Determined by Gas-Liquid Chromatography

The results of the gas-liquid chromatographic study of chickpea flour lipids are shown in Table III and Fig. V.

The major fatty acids found in chickpea flour lipids were linoleic, oleic, and palmitic acid with a content of 55.65, 29.55 and 11.22%, respectively. A small percentage of linolenic and stearic acid was also found with the presence of trace amounts of myristic, palmitoleic, and arachidic acid.

The data indicated that unsaturated fatty acids were the major fatty acids in chickpea flour lipids. The predominant fatty acid of unsaturated

and saturated fatty acids was linoleic and palmitic acid, respectively.

Contents of Total Nitrogen and Phosphorus in Extracted Lipids

Contents of total nitrogen and phosphorus of water-saturated n-butanol extracted lipid were determined as described before. Lipids with and without additional calcium chloride washing were used. The result was shown in Table IV. Value of 0.2927% for total nitrogen and 0.0330% for phosphorus was obtained respectively for lipids without calcium chloride washing, and value of 0.1455% and 0.0201% for lipids with additional calcium chloride washing. It was apparent that calcium chloride washing caused loss of nitrogen- and phosphorus-containing compounds.

Physical and Chemical Properties of Chickpea Oil

The physical and chemical properties of chickpea oil were given in Table V. There was a good agreement on iodine value and unsaponifiable matter between this study and that of Winton and Winton (1935). Bhandari et al. (1950) also reported the similar refractive index and saponification value in their study.

SUMMARY

The purpose of this study was to tentatively fractionate and identify the components of chickpea flour lipids by silicic acid column, thin-layer chromatography and gas-liquid chromatography.

Four different methods were used to extract lipids from chickpea flour: Petroleum ether or ethyl alcohol extraction which was carried out on Goldfish extraction aparatus, acid hydrolysis method and water-saturated n-butanol extraction. Among these, the water-saturated n-butanol extraction was found to be the most effective method for extraction of chickpea flour lipid.

The lipids extracted with water-saturated n-butanol were washed with dilute calcium chloride to remove impurities and non-lipid materials. The purified lipid fraction was further subfractionated into neutral and polar lipids by silicic acid column chromatography, which were identified by thin-layer chromatography on silica gel G plate. Characterization of neutral and polar lipid fraction by TLC showed that triglyceride was predominant in neutral lipids where as lecithin was predominant in polar lipids.

The fatty acid composition of chickpea flour lipid determined by gas-liquid chromatography on diethylene glycol succinate column revealed that the predominant fatty acids of chickpea flour lipid were linoleic, oleic and palmitic at 55.65, 29.55, and 11.22%, respectively. Small amounts of linolenic, stearic and traces of arachidic, palmitoleic, and myristic acids were also found.

It was also found that chickpea oil had a refractive index of 1.5168-1.5175, saponification value 184, iodine value 117; and it contained 0.49% unsaponifiable matter.

TABLE I. LIPIDS EXTRACTED FROM CHICKPEA FLOUR BY DIFFERENT EXTRACTION METHOD

Extraction Method	% Lipid Extracted from Chickpea Flour
Petroleum ether extraction	5.5214
Ethyl alcohol extraction	5.2900
Acid hydrolysis method	5.9613
Water-saturated n-butanol extraction	6.8600

TABLE II. SILICIC ACID COLUMN CHROMATOGRAPHY OF N-BUTANOL EXTRACTABLE LIPIDS IN CHICKPEA FLOUR*

Treatment of Sample	% Total Lipid in Chickpea Flour	% Neutral Lipid in Total Lipid	% Polar-Lipid in Total Lipid	% Recovery
Washed with $CaCl_2$ Solution	4.83	91.09	6.13	97.22
Unwashed	6.86	76.39	19.41	95.80

*The data shown was taken as the average of 5 runs.

TABLE III. FATTY ACID COMPOSITION OF CHICKPEA FLOUR LIPID

Fatty Acid	Percentage of Total Fatty Acid
Saturated Fatty Acid	12.86
Myristic Acid (14:0)	Trace
Palmitic Acid (16:0)	11.22
Stearic Acid (18:0)	1.64
Arachidic Acid (20:0)	Trace
Unsaturated Fatty Acid	87.14
Palmitoleic Acid (16:1)	Trace
Oleic Acid (18:1)	29.55
Linoleic Acid (18:2)	55.65
Linolenic Acid (18:3)	1.94

TABLE IV. CONTENTS OF TOTAL NITROGEN AND PHOSPHORUS IN EXTRACTED LIPIDS*

	Treatment of Sample	
	Washed with $CaCl_2$ Solution	Unwashed
% Nitrogen in Total Lipid	0.1455	0.2927
% Phosphorus in Total Lipid	0.0201	0.0330

*Water-saturated n-butanol extracted lipids.

TABLE V. SOME PHYSICAL AND CHEMICAL PROPERTIES OF CHICKPEA OIL

Refractive Index (at 25°C)	1.5172
Saponification Value	184
Iodine Value	117
Unsaponifiable Matter	0.49%

Fig. I. TLC of neutral lipids extracted by water-saturated n-butanol, washed with $CaCl_2$ solutions followed by fractionation on silicic acid column. From left to right: 1), 4), and 7) neutral lipids with $CaCl_2$ washing, 2) trilinolein, 3) 1,3-diolein, 5) 1,2-diolein, 6) cholesteryl oleate, 8) linoleic acid. Spots 1, 4, 7 were applied at 50 μg level; spots 2, 3, 5 and 6 at 20 μg; spot 8 was applied at 10 μg level. Chromatogram developed with hexane:diethyl ether:acetic acid (70:30:2 by volume); spots visualized by charring with chromic-sulfuric acid. Tentatively identified as: A) sterol ester, B) triglyceride, C) fatty acid, D) 1,3-diglyceride, E) 1,2-diglyceride, F) 1,2-diglyceride.

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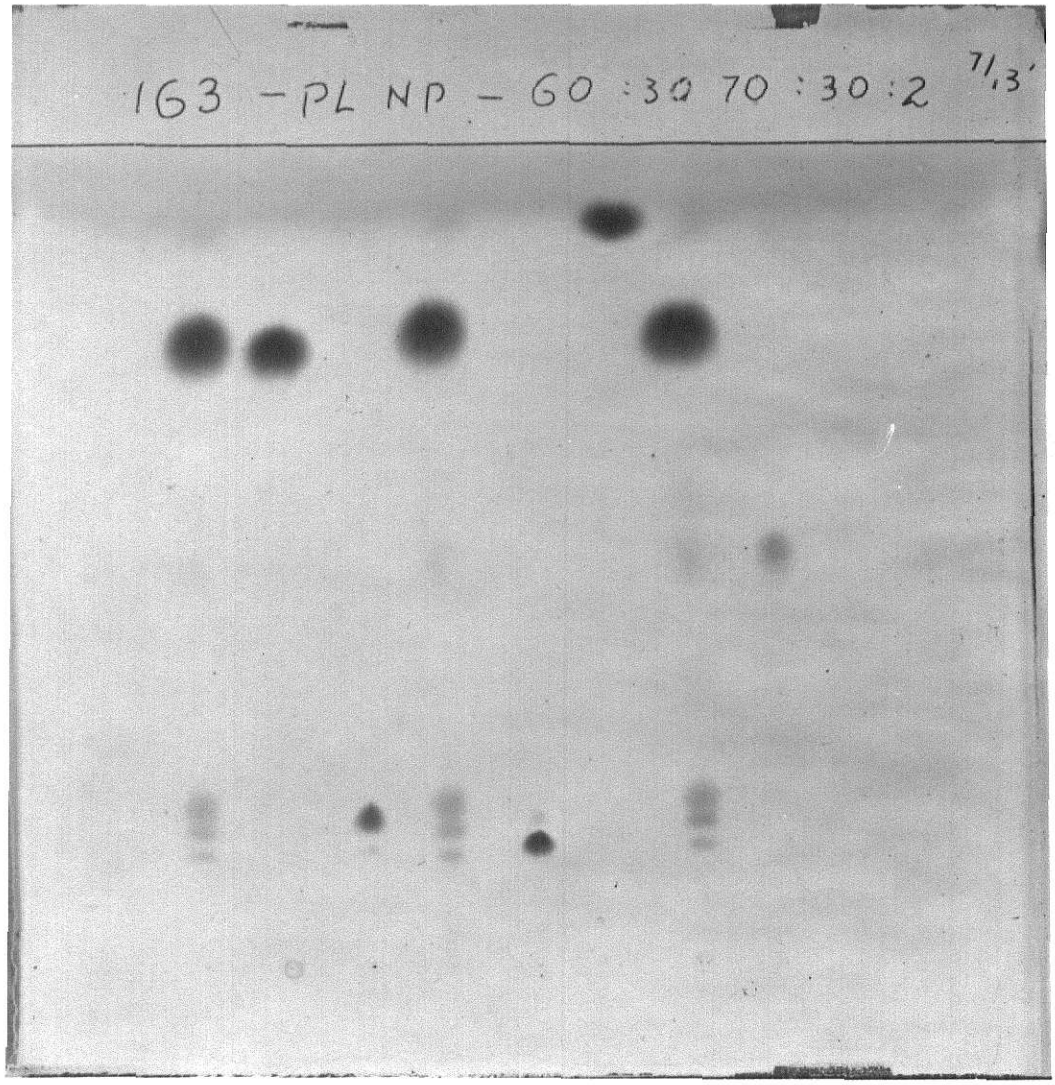

FIG. I

Fig. II. TLC of neutral lipids extracted by water-saturated n-butanol, with and without $CaCl_2$ washing followed by fractionation on silicic acid column. From left to right: 1) trilinolein, 2) 1,3-diolein, 3) neutral lipids with $CaCl_2$ washing, 4) 1,2-diolein, 5) cholesteryl oleate, 6) neutral lipids without $CaCl_2$ washing, 7) linoleic acid. Spots 1, 2, 4, and 5 were applied at 20 μg level; spots 3, and 6 at 50 μg level; spot 7 was applied at 10 μg level. Chromatogram developed with hexane:diethyl ether:acetic acid (70:30:2 by volume); spots visualized by charring with chromic-sulfuric acid. Tentatively identified as: A) sterol ester, B) triglyceride, C) fatty acid, D) 1,3-diglyceride, E) 1,2-diglyceride, F) 1,2-diglyceride.

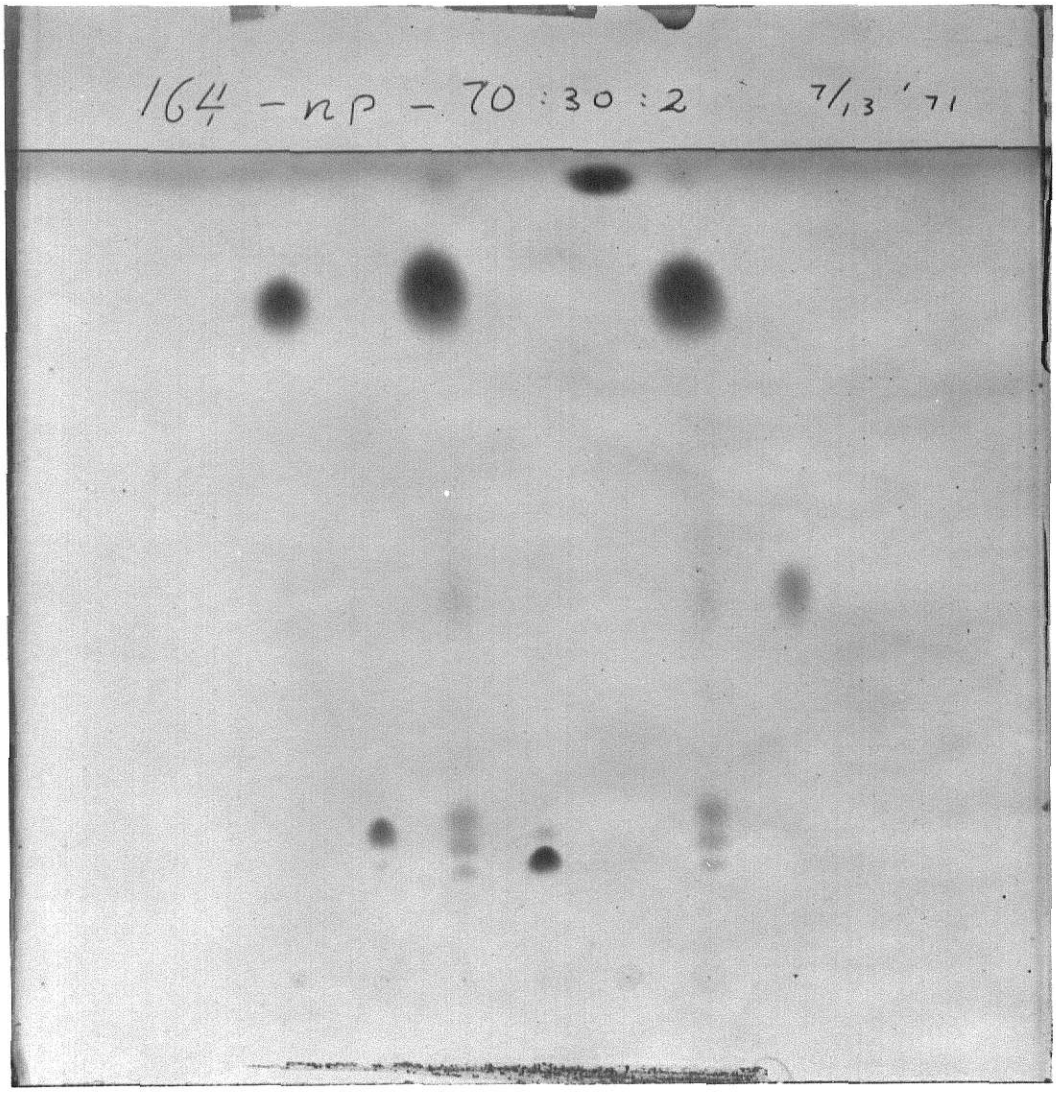

FIG. II

Fig. III. TLC of polar-lipids extracted by water-saturated n-butanol, washed with $CaCl_2$ solutions followed by fractionation on silicic acid column. From left to right: 1) phosphatidyl ethanolamine, 2) polar-lipids with $CaCl_2$ washing, 3) phosphatidyl inositol, 4) polar-lipids with $CaCl_2$ washing, 5) DL-α lecithin (synthetic). Spots 1, 2, 4, and 5 were applied at 50 μg level; spot 3 was applied at 20 μg level. Chromatogram developed with chloroform:methanol:water (60:30:5 by volume); spots visualized by charring with chromic-sulfuric acid. Tentatively identified as: A) possible phospholipids, B) possible glycolipids, C) phosphatidyl ethanolamine, D) lecithin, E) phosphatidyl inositol.

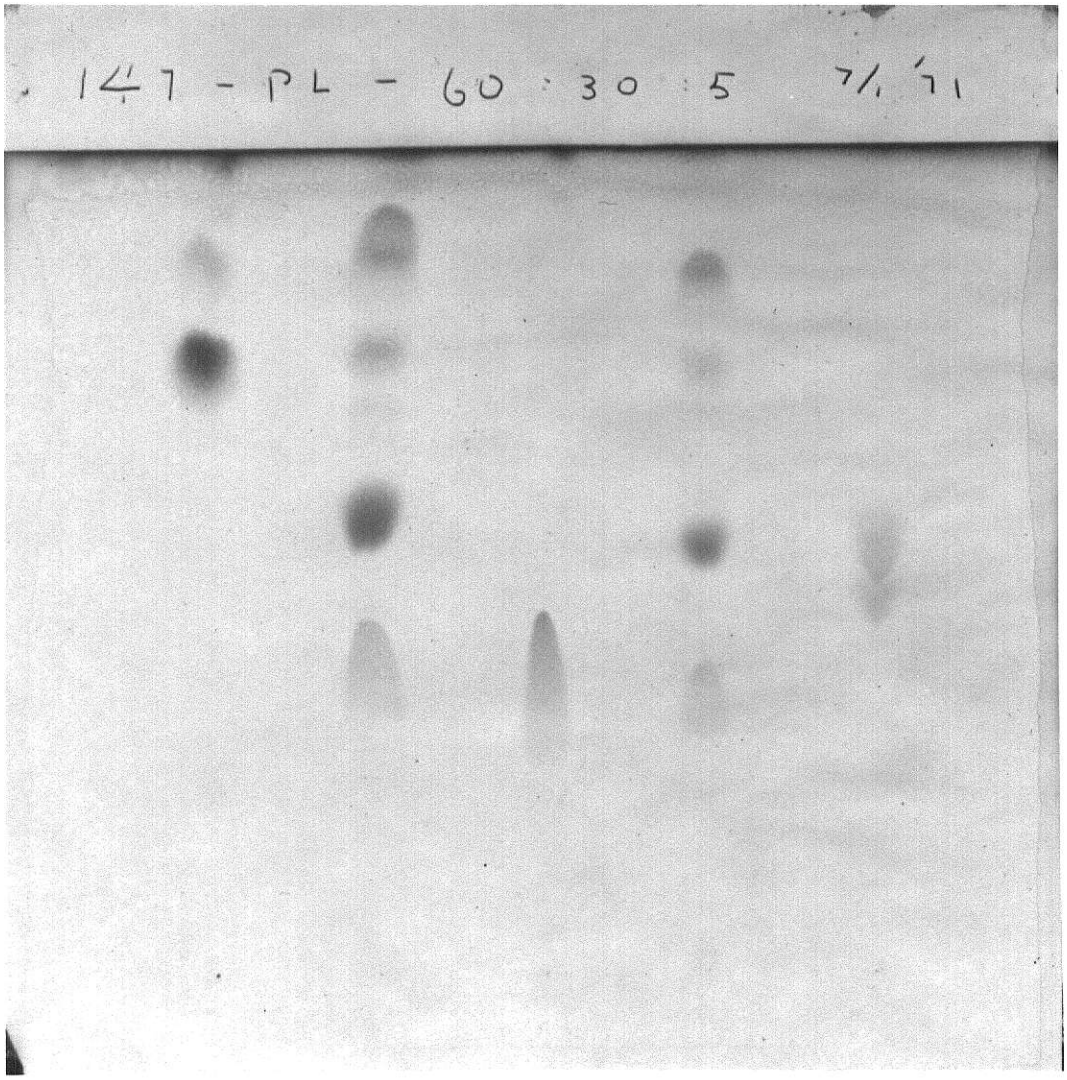

FIG. III

Fig. IV. TLC of polar-lipids extracted by water-saturated n-butanol, with and without $CaCl_2$ washing followed by fractionation on silicic acid column. From left to right: 1) phosphatidyl ethanolamine, 2) polar-lipids with $CaCl_2$ washing, 3) phosphatidyl inositol, 4) DL-α lecithin (synthetic), 5) d (+) raffinose, 6) polar-lipids without $CaCl_2$ washing, 7) sucrose. Spots 1, 2, and 4 were applied at 50 µg level; spots 5 and 7 at 25 µg level; spots 3 and 6 were applied at 20 and 100 µg level, respectively. Chromatogram developed with chloroform:methanol:water (60:30:5 by volume); spots visualized by charring with chromic-sulfuric acid. Tentatively identified as: A) possible phospholipids, B) possible glycolipid, C) phosphatidyl ethanolamine, D) lecithin, E) phosphatidyl inositol, F) sucrose, G) raffinose.

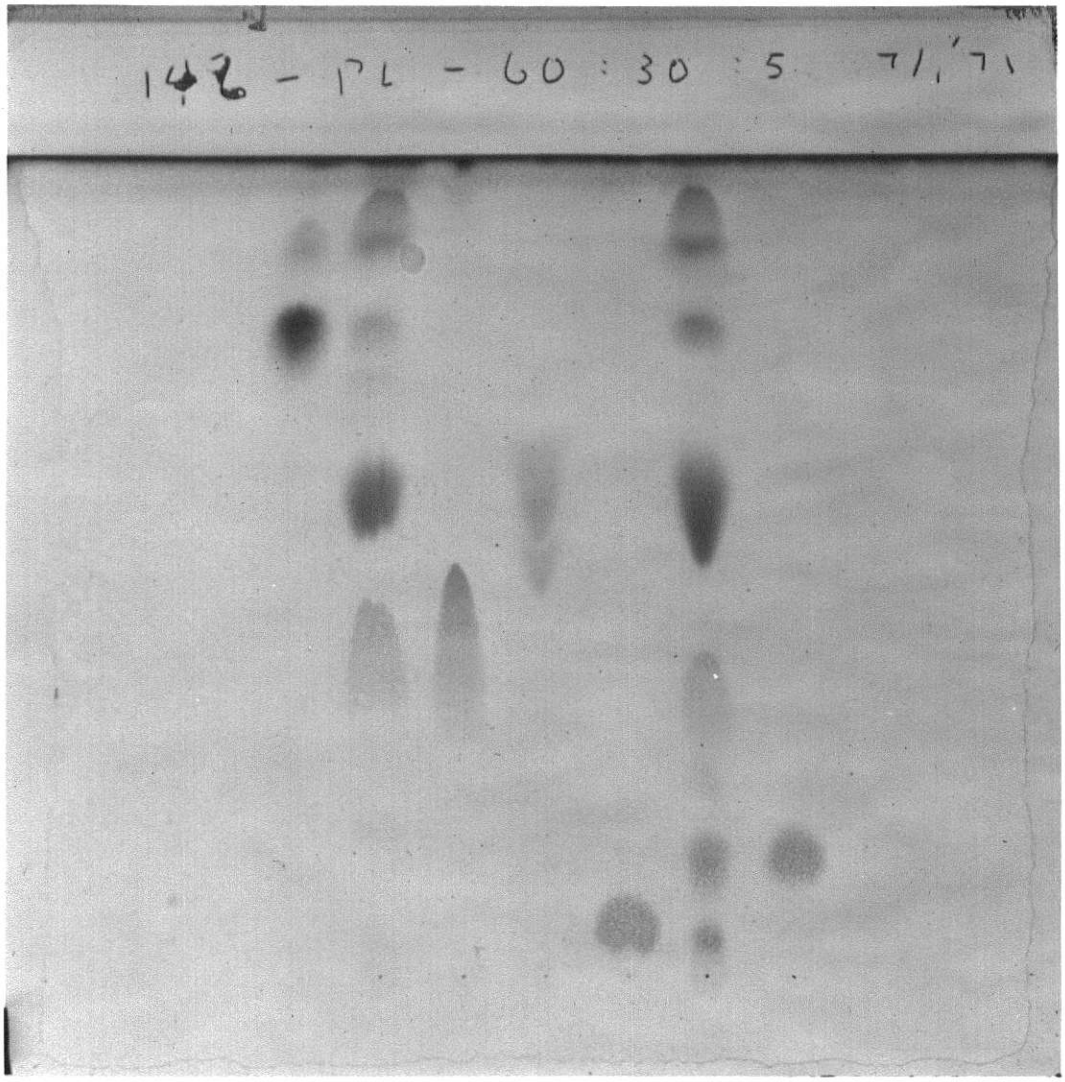

FIG. IV

FIG. V. GAS-LIQUID CHROMATOGRAPHY OF FATTY ACIDS OF CHICKPEA FLOUR LIPID

This chromatogram of fatty acid methyl ester prepared from the chickpea flour lipid sample was produced with Barber-Colman 5000 gas chromatograph fitted with a flame ionization detector. A glass U-tube column 6 feet long with a packing of 7.5% DEGS on 60-80 mesh AW DMCS Chromosorb G was used. The injector heater was operated at 220°C. The carrier gas was argon used at a flow rate of 40 ml./min.

The number beside the peaks indicated the structure of the acids as follows: 18:2 is the designation for an acid containing 18 carbon atoms and having two double bonds or linoleic acid.

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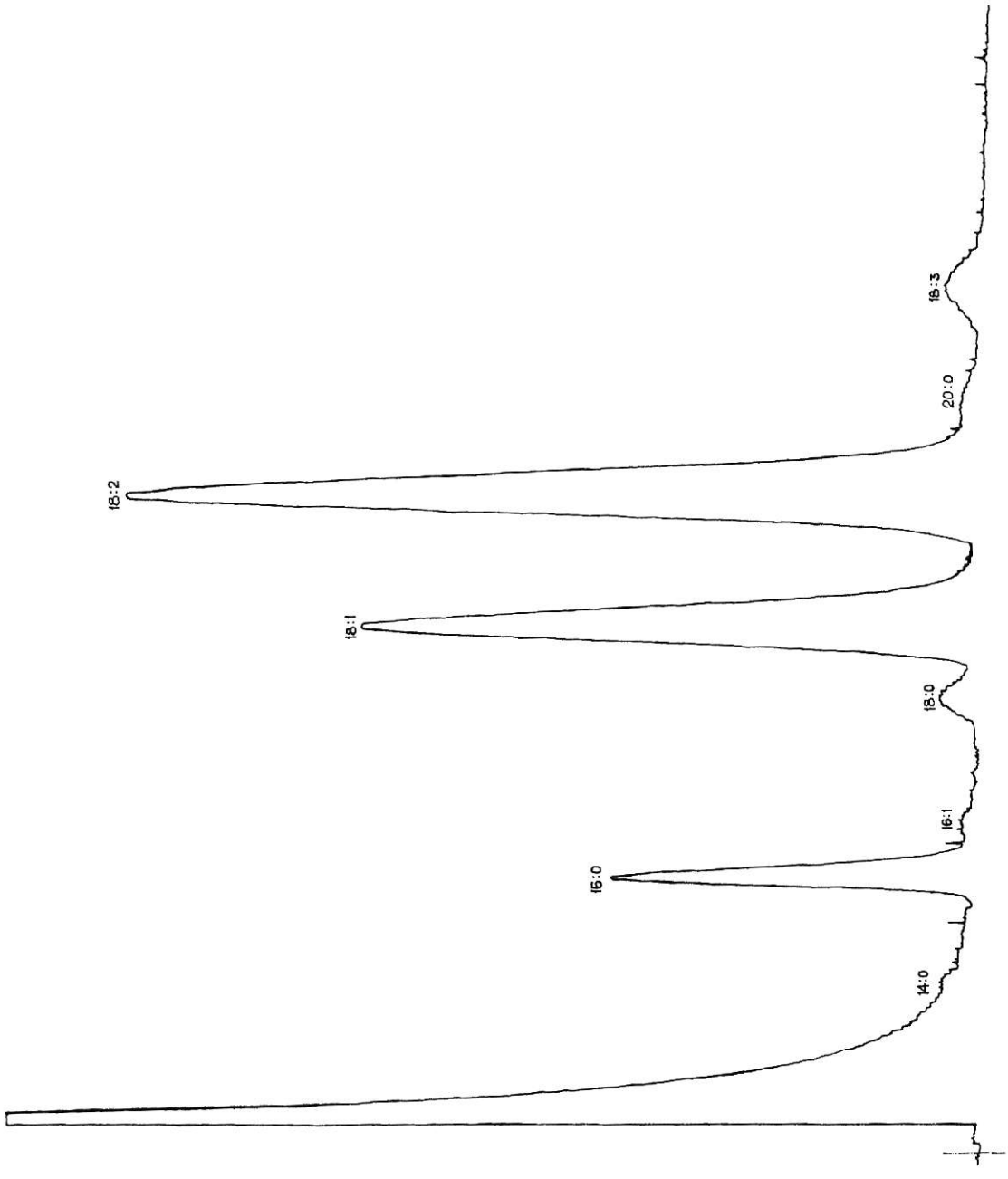

FIG. V GAS-LIQUID CHROMATOGRAPHY OF FATTY ACIDS OF CHICKPEA FLOUR LIPID

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LITERATURE CITED

ABRAMOVA, E. P., CHERNIKOV, M. P. Proteinase inhibitor content in the seeds of some leguminous seeds. Vopr. Pitaniya 23: 13 (1964). Original not seen. Abstracted in Chem. Abstr. 61: 8630e (1964).

ADOLPH, W. H., SHAMMAS, E. I., and HALABY, S. H. The nutritive value of legume proteins and legume-wheat mixed proteins in Near East diets. Food Research 20: 31 (1955).

ALAMO, K. A. Lysine content of broad beans and chickpeas of Chile. Anales Fac. Quim. Farm., Univ. Chile 15: 65 (1963). Original not seen. Abstracted in Chem. Abstr. 63: 1153a (1965).

ALTSCHUL, A. M., editor. Processed Plant protein foodstuffs. Academic Press: New York (1958).

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal Laboratory Methods (7th ed.) The Association: St. Paul, Minnesota (1962).

AMERICAN OIL CHEMISTS' SOCIETY. Official and tentative methods (3rd ed.) Method Ca 6a - 40. American Oil Chemist's Society: Chicago, Illinois. (1958).

AMERICAN OIL CHEMISTS' SOCIETY. Official and tentative methods (3rd ed.) Method Cc 7-25. American Oil Chemists' Society: Chicago, Illinois (1958).

AMERICAN OIL CHEMISTS' SOCIETY. Official and tentative methods (3rd ed.). Method Cd 3-25. American Oil Chemists' Society: Chicago, Illinois (1958).

APPLEBAUM, S. W., MARCO, S., and BIRK, Y. Saponins as possible factors of resistance of legume seeds. J. Agr. Food Chem. 17: 618 (1969).

ASENJO, C. F., and MUNIZ, A. I. Pantothenic acid content of tropical foods. Food Research 20: 47 (1955).

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official methods of analysis (10th ed.) The Association: Washington, D. C. (1965).

AYKROYD, W. R., and DOUGHTY, J. Legumes in human nutrition. Food and Agriculture Organization of the United Nations: Rome (1964).

BANERJEE, D. K., and CHATTERJEA, J. B. Folic acid activity of Indian dietary articles and the effect of cooking on it. Food Technol. 18: 1081 (1964).

BHANDARI, P. R., BOSE, J. L., and SIDDIQUI, S. Studies in the constituents of chana (Cicer arietinum, L.). _____ Part III. Chemical examination of the fixed oils from chana and Kabuli chana (ordinary and white varieties). J. Sci. Ind. Research (India) 9B: 60 (1950).

BHIDE, N. V., and SAHASRABUDDHE, D. L. The enzymes present in germinating seeds. J. Univ. Bombay 12A: 81 (1943a). Original not seen. Abstracted in Chem. Abstr. 38: 17656 (1944).

BHIDE, N. V., and SAHASRABUDDHE, D. L. Enzymes present in germinating seeds. II. Optimum pH for enzymes from germinating bajri and gram. Rasayanam 1: 258 (1943b). Original not seen. Abstracted in Chem. Abstr. 38: 42809 (1944).

BORCHERS, R., and ACKERSON, C. W. Trypsin inhibitor. IV. Occurrence in seeds of the Leguminosae and other seeds. Arch. Biochem. 13: 291 (1947).

BREGOFF, H. M., ROBERTS, E., and DELWICHE, C. C. Paper chromatography of quaternary ammonium bases and related compounds. J. Biol. Chem. 205: 565 (1953).

CARRASCO, M. O. M. Evaluation of riboflavine in seeds of germinated legumes. Anales fac. farm. y bioquim., Univ. nacl. mayor San Marcos 5: 136 (1954). Original not seen. Abstracted in Chem. Abstr. 51: 3756e (1957).

CHANDRA, S., and ARORA, S. K. An estimation of protein, ascorbic acid, and mineral matter content in some indigenous and exotic varieties of gram (Cicer arietinum L.). Curr. Sci. (India) 37: 237 (1968).

CHERNIKOV, M. P., ABRAMOVA, E. P., LYAIMAN, M. E., CHERNIKOVA, L.G., SHULYAK, A. I., and CHEBOTAREV, A. K. Inhibitors of proteinases contained in seeds of some plants, their properties and effect on protein assimilation. Vestn. Akad. Med. Nauk. SSSR 21: 57 (1966). Original not seen. Abstracted in Chem. Abstr. 67: 80169h (1967).

COLOBRADO, V., SANAHUJA, J. C., and RIOS, D. S. Determination of amino acids in the proteins of various edible legumes in Argentina. Rev. Farm. (Buenos Aires) 101: 58 (1959). Original not seen. Abstracted in Chem. Abstr. 54: 9151g (1960).

CORREA, A. M. N., ROSENTHAL, F. R. T., and TOLMASQUIM, E. Chickpea (Cicer arietinum L.) starch. II. Swelling power and solubility. Anals Acad. Brasil Cienc. 37: 241 (1965). Original not seen. Abstracted in Chem. Abstr. 64: 19954c (1966).

COURTOIS, J., and PEREZ, C. Phytase VIII. Inositol hexaphosphate content and phytase activity of various seeds. Bull. soc. chim. biol. 29: 195 (1947). Original not seen. Abstracted in Chem. Abstr. 42: 8223i (1948).

COZZI, A. Chemical and physical characters of the chickpeas of the Italian East Africa. Agr. Coloniale 33: 44 (1939). Original not seen. Abstracted in Chem. Abstr. 38: 55354 (1944).

DAFTARY, R. D. and POMERANZ, Y. Changes in lipid composition in maturing wheat. J. Food Sci. 30: 577 (1965).

DAFTARY, R. D., WARD, A. B., and POMERANZ, Y. Distribution of lipids in air-fractionated flours. J. Food Sci. 31: 897 (1966).

DAMODARAN, M., and SUBRAMANIAN, S. S. Amide synthesis in plants. IV. Aspartase in germinating seedlings. Proc. Indian Acad. Sci. 27B: 47 (1948). Original not seen. Abstracted in Chem. Abstr. 42: 4649i (1948).

DATE, W. B. Antioxidant property of legume flours. Science and Culture (India) 21: 106 (1955). Original not seen. Abstracted in Chem. Abstr. 50: 10292i (1956).

DIAZ, C. A. T., and WHITE, P. L. Food value of legumes consumed in Peru. Actas y trabajos congr. Peruanoquim 356 (1953). Original not seen. Abstracted in Chem. Abstr. 51: 5324h (1957).

DITTMER, J. C., and LESTER, R. L. A simple specific spray for the detection of phospholipids on thin-layer chromatograms. J. Lipid Res. 5: 126 (1964).

EL-HANAFY, A. R. S., and TAHA, M. I. An arabinoglucoglycan in chickpea seed. J. Org. Chem. 28: 1559 (1963).

ESH, G.C., and SOM, J. M. Nutritional survey on available food materials. III. Nutritive value of pulses. Indian J. Physiol. and Allied Sci. 6: 61 (1952). Original not seen. Abstracted in Chem. Abstr. 46: 10332b (1952).

FABRIANI, G., and SPADONI, M. A. Thiamine in Italian vegetables and fruits. Quaderni nutriz. 10: 88 (1947). Original not seen. Abstracted in Chem. Abstr. 43: 6753g (1949).

GANAPATI, R., SWAMINATHAN, M. C., TASKAR, A. D., and RAO, K. S. Feeding trials with vegetable protein foods. Ind. J. Med. Res. 49: 306 (1961).

GIRAL, F., and ALVAREZ, C. S. Vitamin C content of Mexican legumes and vegetables. Ciencia (Mex.) 4: 66 (1943).

GIRAL, J., and CASTILLO, M. T. Determinations of copper in Mexican food. Mem. congr. cient. Mex., IV Centenario Univ. Mex. 2: 232 (1953). Original not seen. Abstracted in Chem. Abstr. 49: 1981c (1955).

GOLDBERG, L., THORP, J. M., and SUSSMAN, S. A survey of vitamins in African foodstuffs. IV. The thiamine content of beans and other legumes. S. African J. Med. Sci. 10: 87 (1945). Original not seen. Abstracted in Chem. Abstr. 40: 74219 (1946).

GUGGENHEIM, K., and SZMELCMAN, S. Protein-rich mixture based on vegetable foods available in Middle Eastern countries. J. Agr. Food Chem. 13: 148 (1965).

GUTTKIAR, M. N., SHAH, DAMAYANTI H., ADHIKARI, H. R., AMBERGAOKAR, S. D., and RAO, M. V. R. Studies on the nutritive value of Indian Foodstuffs. III. Magnesium and copper contents of some foods. J. Nutr. and Dietet. 3: 4 (1966).

HADI, S. M. Quantitative changes in necleic acids and nucleases during germination of Cicer arietinum L. Indian J. Biochem. 3: 203 (1966). Original not seen. Abstracted in Chem. Abstr. 66: 17050b (1966).

HADI, S. M., KRISHNA, MURTI, C. R. Purification and properties of ribonuclease, 3-nucleotidase and phosphomonoesterase from Cicer arietinum L. seedlings. Indian J. Biochem. 5: 53 (1968).

HARRIS, W. D., and POPAT, P. Determination of the phosphorus content of lipids. J. Am. Oil Chemists' Soc. 31: 124 (1954).

HOFSTETTER, H. H., SEN, N., and HOLMAN, R. T. Characterization of unsaturated fatty acids by gas-liquid chromatography. J. Am. Oil Chemists' Soc. 42: 537 (1965).

HOSEL, W., and BARZ, W. Flavonoids of Cicer arietinum L. Phytochemistry 9: 2053 (1970).

IENGAR, N. G. C., JAYARAMAN, V., and RAU, Y. V. S. Thiamine contents of Indian foodstuffs. II. Thiamine content of a few leguminous seeds during germination. Ann. Biochem. and Exptl. Med. (India) 15: 49 (1955). Original not seen. Abstracted in Chem. Abstr. 50: 6596i (1956).

INTENGAN, C. L., CONCEPCION, I., SALUD, R. C., MANALO, J., ROSARIO, I. D., GOMEZ, R., ARZAGA, V., and ALEJO, L. G. Composition of Philippine foods. II. Philippine J. Sci. 83: 187 (1954).

JAMIESON, G. R., and REID, E. H. The analysis of oils and fats by gas chromatography. J. Chromatog., 17: 230 (1965).

KANDE, J. Nutritional value of two leguminous seeds, chickpeas, Cicer arietinum L., and lentils, Lens esculenta. Ann. Nutr. Aliment 21: 45 (1967). Original not seen. Abstracted in Chem. Abstr. 67: 30232c (1967).

KHAN, A. W., SAMULA, B., NUR-UD-DIN, and CHUGHTAI, I. D. Nutritive value of foodstuffs. III. Cereals and pulses. Pakistan J. Sci. Research 7: 115 (1955). Original not seen. Abstracted in Chem. Abstr. 50: 2884h (1956).

KLOPFENSTEIN, W. E. On methylation of unsaturated acids using boron trihalide-methanol reagents. J. Lipid Res., 12: 773 (1971).

LAIN, E. R., GALLEGO, J. R. M., and CORTES, J. O. Amino acids in seeds of legumes cultivated in Spain. Rev. Nutri. Animal (Madria) 1: 24 (1963). Original not seen. Abstracted in Chem. Abstr. 62: 1012f (1965).

LAL, S. B. Microbiological assay of amino acids in gram and ragi. Ind. J. Med. Res. 38: 131 (1950).

LEONOV, G. B. Variations in protein contents in seeds of some Cicer arietinum L. varieties. Tr. Khim. Prir. Soedin Gos. Univ. 6: 95 (1965) (pub. 1966). Original not seen. Abstracted in Chem. Abstr. 67: 789x (1967).

Leonov, G. B. Chromatographic separation of proteins from Cicer arietinum L. (chickpea) cotyledons. Tr. Khim. Prir. Soedin. 7: 77 (1968). Original not seen. Abstracted in Chem. Abstr. 73: 8465e (1970).

LEPAGE, M. The separation and identification of plant phospholipids and glycolipids by two-dimensional thin-layer chromatography. J. Chromatog. 13: 99 (1964).

LISANTI, L. E. Distribution of cyanase activity in plants. I. Cyanolytic activity in Cicer arietinum L. Ann. Sper. Agrar. 17: 45 (1963). Original not seen. Abstracted in Chem. Abstr. 64: 7037d (1966).

MANGOLD, H. K. Thin-layer chromatography of lipids. J. Am. Oil Chemists' Soc. 38: 708 (1961).

MASSIEU, G. H., GUZMAN, J. G., CRAVIOTO, R. O., and DELLA TORRE, J. C. Essential amino acid content in several Mexican Seeds. Ciencia (Mex.) 10: 142 (1950).

MECHAM, D. K., and MOHAMMAD, A. Extraction of lipids from wheat products. Cereal Chem. 32: 405 (1955).

MEHLENBACHER, V. C., editor. The analysis of fats and oils. The Garrard Press: Champaign, Illinois (1960).

MORTON, R. K. Separation and purification of enzymes associated with insoluble particles. Nature 166: 1092 (1950).

NAIR, P. M., and VAIDYANTHAN, C. S. Tryptophan synthetase in Plants. Arch. Biochm. Biophys. 93: 262 (1961).

NAIR, P. M., and VAIDYANTHAN, C. S. A study of the purification and properties of tryptophan synthetase of Bengal gram (Cicer arietinum L.). Arch. Biochem. Biophys. 104: 405 (1964).

NAZIR, D. J., and MAGAR, N. G. Tocopherol content of pulses. Indian J. Chem. 1: 278 (1963).

NEISSER, M. R. Content in tryptophan of broad beans and chickpeas of Chile. Anales Fac. Quim. Farm., Univ. Chile 15: 65 (1963, 1964). Original not seen. Abstracted in Chem. Abstr. 63: 1153b (1965).

NIGAM, V. N., and GIRI, K. V. Sugar in legumes. Can. J. Biochem. and Physiol. 39: 1847 (1961).

PANT, R., and KAPUR, A. S. The soluble carbohydrates of some Indian legumes. Naturwissenschaften 50: 94 (1963). Original not seen. Abstracted in Chem. Abstr. 58: 10512a (1963).

PANT, R., and KAPUR, A. S. A comparative study of the chemical composition and nutritive value of some common Indian pulses and soybean. Ann. Biochem. Exptl. Med. (Calcutta) 23: 457 (1963). Original not seen. Abstracted in Chem. Abstr. 60: 11286h (1964).

PARIHAR, D. B. Changes in the saccharides of stored pulses. Naturwissenschaften 41: 427 (1954). Original not seen. Abstracted in Chem. Abstr. 49: 10445f (1955).

PATEL, R. D., PATEL, R. P., and PATEL, R. S. Specific rotation of cereal and legume starches. Cereal Chem. 37: 500 (1960).

POMERANZ, Y., CHUNG, O., and ROBINSON, R. J. The lipid composition of wheat flours varying widely in breadmaking potentialities. J. Am. Oil Chemists' Soc. 43: 45 (1966).

PRIVETT, O. S., and BLANK, M. L. A method for the structural analysis of triglycerides and lecithins. J. Am. Oil Chemists' Soc. 40: 70 (1963).

ROUSER, G., GALLI, C., and LIEBER, E. Analytical fractionation of complex lipid mixtures: DEAE cellulose column chromatography combined with quantitative thin layer chromatography. J. Am. Oil Chemists' Soc. 41: 836 (1964).

ROYCHOWDHURY, S. P., KHAN, J. A., and BOSE, K. N. Minor mineral contents of some foods. J. Proc. Inst. Chemists (India) 34: 89 (1962). Original not seen. Abstracted in Chem. Abstr. 57: 11607c (1962).

RUDRA, M. N. Studies in vitamin C. V. The vitamin C content of some germinated cereal and pulses. J. Indian Chem. Soc. 15: 191 (1938). Original not seen. Abstracted in Chem. Abstr. 32: 75213 (1938).

RUDRA, M. N. Ascorbic acid content of recently harvested cereals and legumes. Nature 152: 78 (1943).

SANZANA, H. H., and BODOY, H. C. Threonine, valine, and methionine in chickpeas and lentils. Rev. Real Acad. Cienc. Exactas, Fis. Natur. Madrid 61: 655 (1967). Original not seen. Abstracted in Chrm. Abstr. 68: 67873j (1968).

SARIN, J. L., and QURESHI, M. H. Starch form gram. Ind. Eng. Chem. 30: 1318 (1938).

SCHLACK, D. G. Microbiological evaluation of methionine, threonine, leucine and isoleucine in Chilean chickpeas (Cicer arietinum L.). An. Fac. Quim. Farm., Univ. Chile 18: 218 (1966) (pub. 1968). Original not seen. Abstracted in Chem. Abstr. 68: 112139n (1968).

SHEHATA, N. A., and Fryer, B. A. Effect on protein quality of supplementing wheat flour with chickpea flour. Cereal Chem. 47: 663 (1970).

SIMS, R. P. A., and LAROSE, J. A. G. The use of iodine vapor as a general detecting agent in the thin layer chromatography of lipids. J. Am. Oil Chemists' Soc. 39: 232 (1962).

SOHONIE, K., and BHANDARKAR, A. P. Trypsin inhibitors in Indian foodstuffs: Part I - Inhibitors in vegetables. J. Sci. Ind. Research (India) 13B: 500 (1954).

SRIKANTIA, S. G., and GOPOLAN, C. Clinical trials with vegetable protein foods in Kwashiorkor. Ind. J. Med. Res. 48: 637 (1960).

SRIVASTAVA, H. C., HARSHE, S. N., GHARIA, M. M., MUDIA, G. P., and AHMENDABAD. Physicochemical studies on some starches. Die Starke 22: 162 (1970).

STAHL, E., editor. Thin-layer chromatography (2nd ed.). Springer-Verlag: New York (1969).

TALWALKAR, R. T., GARG, N. K., and MURTI, C. R. K. Properties of phosphatides prepared from rice bran, Cicer arietinum L., and Phaseolus aureus. Indian J. Biochem. 3: 124 (1966). Original not seen. Abstracted in Chem. Abstr. 65: 17257g (1966).

TOLMASQUIM, T., ZAMITH, M. A., ROSENTHAL, F. R. T., and TOLMASQUIM, E. Chickpea (Cicer arietinum L.) starch. I. Barbender viscosity. Anals Acad. Brasil Cienc. 37: 235 (1965). Original not seen. Abstracted in Chem. Abstr. 64: 19953h (1966).

TORRES, G. B., and BODOY, H. C. Isoleucine, leucine, and phenylalanine content in Chilean legumes. Rev. Real Acad. Cienc. Exactas, Fix. Natur. Madrid 61: 667 (1967). Original not seen. Abstracted in Chem. Abstr. 68: 67911v (1968).

TSEN, C. C., LEVI, I., and HLYNKA, I. A rapid method for the extraction of lipids from wheat products. Cereal Chem. 39: 195 (1962).

URRUTIA, J. A. Photocolorimetric determination of nicotinic acid in several national breadstuffs. Actas y trabajos congr. Peruano quim, 3° Congr. (Lima, Peru) 2: 471 (1949). Original not seen. Abstracted in Chem. Abstr. 45: 5835f (1951).

WAGNER, H., HORHAMMER, L., and WOLFF, P. Thin-layer chromatography of phosphatides and glycolipids. Biochem. Ztschr. 334: 175 (1961).

WINTON, A. L., and WINTON, K. B., editor. The structure and composition of food, Vol. II. Wiley: New York (1935).

WONG, E., MORTIMER, P. I., and GEISSMAN, T. A. Flavonoid constituents of Cicer ariteinum L. Phytochemistry 4: 89 (1965).

A STUDY OF LIPIDS OF CHICKPEA

by

LEH-YEU LING

B. S., Taiwan Provincial Chung-Hsing University (China), 1966

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The present investigation was undertaken to separate and identify the lipids from chickpea flour and to study some of their physical and chemical properties.

Chickpea flour lipids were extracted by using four different methods. (1) Petroleum ether and (2) ethyl alcohol extraction were carried out on Goldfish extraction apparatus. (3) Acid hydrolysis using concentrated hydrochloric acid: the hydrolysate was subsequently extracted by ether and petroleum ether. (4) The water-saturated n-butanol extraction was done in the Stein Mill. The last method was found to be the most efficient to extract the lipids from chickpea flour.

Lipids extracted by water-saturated n-butanol were washed by dilute calcium chloride solution in order to remove the non-lipid materials. They were further fractionated into neutral and polar lipids by silicic acid column chromatography. These two fractions were subfractionated and identified by thin-layer chromatography on silica gel G plates.

TLC chromatograms showed that triglyceride was the major component of the neutral lipids whereas lecithin was the major component of the polar lipids.

The fatty acids composition of chickpea flour lipids were determined by gas-liquid chromatography. GLC chromatograms revealed that major fatty acids of chickpea flour lipids were linoleic, oleic, and palmitic acid, 55.65%, 29.55%, and 11.22% respectively. Stearic acid, linolenic acid were found in small amount; and myristic, palmitoleic, and arachidic acid were in trace.

Contents of the total nitrogen and phosphorus determined by micro-Kjeldahl procedure and Harris and Popat method were found 0.1455 and 0.0201% of the total lipid respectively.

The following physical and chemical properties were used to characterize the lipid extracted from chickpea flour, refractive index, saponification value, and unsaponifiable matter.